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Precision of autoantibody assays in clinical diagnostic laboratories: What is the reality?

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ABSTRACT

Background: ISO 15189 accreditation remains a challenge for specialized laboratories. In the field of autoimmunity, beside the crucial problem of absence of standardization, laboratories have to manage the analytical performances of the large panel of assays in terms of sensitivity and specificity, but also on their measurement precision for which no reference values are available on biorepositories.

Methods: As an initiative of the French EASI (European Autoimmunity Standardization Initiative) group, French clinical diagnostic laboratories were requested to participate in a survey aiming to analyze the coefficients of variation (CVs) of intra-run and inter-run variability obtained with assays quantifying 14 different autoantibodies. Two performance goals corresponding to the 90th percentile and the 50th percentile (lowest CV values reached by 90% and 50% of laboratories respectively) defined for three levels of concentration were calculated. The impact on the assay performances of the number of measurements, of the nature of the internal quality control (IQC) and the type of immunoassay, was also analyzed.

Results: 414 and 616 values of intra-run and inter-run CVs were collected, respectively. The 50th percentile performance goals were comprised between 1.0% and 8.9% for the intra-run CVs, and between 1.8% and 14.6% for the inter-run CVs. At 90th percentile, the performance goals were comprised between 3.2% and 13.5% for the intra-run CVs, and between 7.3% and 30.8% for the inter-run CVs. CVs calculated from 10 values were similar to those obtained from more values. Higher imprecision was observed when the antibody levels of the IQC was lower than 2 fold the positive threshold. Commercial IQCs gave lower CVs than IQCs derived from patient samples.

Conclusion: Our results allow proposing some acceptability limits for the precision performances of the autoantibody assays, compatible with the reality of life in diagnostic laboratories and clinical care.

1. Introduction

Specialized laboratories have to take up the challenge of applying for and maintaining ISO 15189 accreditation, with specific difficulties

relative to the laboratory equipment, the pre-examination phase and examination procedures [1]. In the field of autoimmunity, the laboratories have to face the crucial issue of the absence of assay standardization. This is linked to the nature of the antigens, the immunoglobulin

Abbreviations: ALBIA, addressable laser bead immunoassay; B2GP1, β 2 glycoprotein 1; CCP, cyclic citrullinated peptides; CDL, clinical diagnostic laboratories; CL, cardiolipin; CLIA, chemiluminescent immunoassay; CV, coefficient of variation; dsDNA, double stranded-DNA; ELISA, enzyme-linked immunosorbent assay; FEIA, fluorescent-enzyme immunoassay; GBM, glomerular basement membrane; IQC, internal quality control; MPO, myeloperoxidase; PR3, proteinase 3; RF, rheumatoid factor; TG, thyroglobulin; TPO, thyroperoxidase; TSH-R, thyroid stimulating hormone (TSH)-receptor; TTG, tissue transglutaminase

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Fig. 1. (A) Pre-defined Excel sheet sent to 88 French clinical diagnostic laboratories. The table was ready to be completed for the different items by unchangeable drop-down menus. (B) Number of coefficients of variation collected for each autoantibody assay.

conjugate, the methods of detection and even the units of the results [2]. Consequently, laboratories have to manage the analytical performance of the large panel of assays available on the market [3]. The evaluation of such performances involves not only the clinical relevance of the assays in terms of sensitivity and specificity, but also their measurement precision. Precision of measurement is the extent to which repeated measurements of the same sample give similar results. It is expressed as standard deviation, variance or, more often, coefficient of variation (CV). Precision is measured for repeatability conditions of measurement (also called intra-serial, within-run or intra-run precision) that includes the same measurement procedure, operator, measuring system, operating conditions, time, and location. Precision is also measured for intermediate conditions of measurement (or inter-serial, between run, within-lab or inter-run precision) that includes the same measurement procedure and location, and replicate measurements on the same or similar objects over an extended period of time, and, as such, may include other conditions involving changes that include other lots of reagents, calibrations, calibrators, operators, and measuring systems. Reproducibility conditions of measurement also include different locations, operators, measuring systems or procedures [4]. While external quality control is used to determine reproducibility, the use of internal quality controls (IQC) allow the calculation of precision, both intra- and inter-run precision which are essential for detecting errors during the analytical phase. This helps to ensure day-to-day consistency of the process and determine whether patient results are reliable enough to be communicated.

The interpretation of precision data obtained in clinical diagnostic laboratories (CDL), *i.e.*, the decision to consider them as acceptable or not, remains challenging in the field of autoimmunity. To date very few reference values have been defined for autoantibody assays and no reference is available on-line or published. Such reference books exist for different specialties, mainly for clinical chemistry [5,6] or hematology [7]. Only some reference CVs may be found for anti-

thyroperoxidase (TPO), anti-thyroglobulin (TG) antibodies and rheumatoid factor (RF) assays [8,9]. The mere absence of reference values for autoantibody diagnostics is partly due to the absence of reference materials in autoimmunity [10]. The Federal Drug Administration (FDA) published guidelines for companies developing diagnostics assays [11], giving global rules and analytical objectives to reach. However, these recommendations are not specific to the autoantibody measured neither to the method of detection used by each assay.

Considering the complexity of the field, the European Autoimmunity Standardization Initiative (EASI) has been formed to harmonize autoantibody diagnostics [12]. The objective of this study was to elaborate a reference support in autoimmunity that could be used in routine practice and for the accreditation of CDL. As an initiative of the French EASI group, a large number of French CDL were requested to participate in a survey aiming to analyze the CVs of intra-run and inter-run precision obtained for assays quantifying 14 different autoantibodies. The results, obtained from 32 specialist laboratories, allow the proposal of acceptability limits for the performances of the autoantibody assays, allowing accurate clinical interpretation of the test and being compatible with the diagnostic laboratories' reality.

2. Material and methods

2.1. Selection criteria of autoantibody assays

The assays included in this study were selected according to the following criteria: quantitative autoantibody assays for which the quantitation is of clinical relevance and performed in a large number of laboratories. So the assays studied were: IgG and IgM isotypes for anti-beta 2 glycoprotein 1 (B2GP1) and anti-cardiolipin (CL) autoantibodies, IgG isotype for anti-myeloperoxidase (MPO-ANCA), anti-proteinase 3 (PR3-ANCA), anti-glomerular basement membrane (GBM), anti-double stranded-DNA (dsDNA), anti-thyroglobulin (TG), anti-thyroperoxidase

Number of measurements used for CVs calculation

Fig. 2. Combined results of intra- and inter-run coefficients of variation according to the number of measurements used for their determination. No statistical difference was observed between the 4 groups.

(TPO), anti-TSH-receptor (TSH-R), and anti-cyclic citrullinated peptides (CCP), IgA isotype for anti-tissue transglutaminase (TTG) auto-antibodies and IgM isotype for rheumatoid factor (RF); the latter, however, is also often measured by nephelometry/turbidimetry and these assays are not specific for IgM isotype only [13].

2.2. Data collection

A participation invitation for this study was sent by email in March 2018 to 88 French CDL, 68 being public hospital laboratories and 20 private CDL. To ensure uniformity of the responses, this e-mail was accompanied by an Excel sheet, ready to be completed for predefined items by fixed drop down menus. Fig. 1A depicts the data collected. In addition to the analyte, for both intra- and inter-run CVs, information was collected on the type of immunoassay, the type of automation (manual, semi- or full automation), the number of values used for the CV calculation, the level of IQC according to the positive threshold (low: less than 2 fold, medium: between 2 and 5 fold, high: superior to 5 fold) and the nature of the sample used as IQC (patient sample prepared in the CDL, kit-related commercial control, or kit-independent commercial control). Each line was to be completed for each analyte and level of IQC. It was possible to complete as many lines as there were controls for the respective analyte. A 5 weeks deadline was given to complete the file and a reminder email was sent 10 days before the end of the study. The questionnaire did not ask for information about the time frame when measurements for intra- and inter-run CV's were done.

2.3. Data analysis

Prior to analysis, CV calculated from less than 5 measurements were excluded. In addition, reported CVs below 0.1% or above 80% were excluded.

Criteria for acceptable performance were defined for each auto-antibody assay from data of CVs sent by participating laboratories. According to the model used by the French Society of Biochemistry [6] (SFBC), we fixed two performance goals corresponding to the 90th percentile and the 50th percentile (median) from CVs distribution. The

first target is the lowest CV value reached by 90% of laboratories and the second by 50% of laboratories. These performance goals were defined for the 3 levels of autoantibodies.

In addition, we analyzed the impact of the number of measurements, the nature of the IQC used, as well as the method (type of immunoassay and automation), on the assay performance.

2.4. Statistical analysis

Prism-GraphPad™ software was used to perform different statistical analysis. Unpaired *t*-test, Mann-Whitney test and one-way ANOVA with multiple comparisons (Turkey's multiple comparison test) were used.

3. Results

The overall response rate of the invited laboratories was 36% (32/88). 20% of private laboratories (4/20) and 41% of hospital laboratories (28/68) participated, allowing collection of 440 values of intra-run CV (25 values were excluded, leaving 414 for analysis) and 654 values of inter-run CV (38 values were excluded leaving 616 for analysis). A mean of 34 values of CVs was provided by the participating laboratories. 41% of the assays were declared accredited by laboratories at the time of the data collection, according to the ISO 15189 guidelines.

The number of CVs obtained for each analyte is depicted in Fig. 1B. It varied from 7 to 53 and from 14 to 68 values per analyte for the CVs of intra-run and inter-run precision, respectively. The highest numbers of collected data were for anti-CCP and RF. The lowest numbers of collected CVs were for anti-TSH-R autoantibody assays.

3.1. Impact of the number of measurements on CV results

We first analyzed the results of CVs according to the number of measurements used for their determination (5–10, 11–20, 21–30 or > 30 values) (Fig. 2). No significant difference was observed between the 4 groups (one-way ANOVA) when considering all data (intra- and inter-run CVs).

Fig. 3. Levels of CVs according to the source of IQC used (commercial control different from the manufacturer of the assay, commercial control included in the kit or patients' serum or plasma derived samples). Lower CVs of intra-run variation were obtained with the commercial controls. NS: $P > 0.05$, ****: $P \leq 0.0001$.

3.2. Impact of the source of the quality controls on CV results

Different types of samples may be used as IQC to perform the measurements allowing the determination of the intra- and inter-run CVs. They may be provided by the manufacturer of the respective immunoassay. Reference values are usually provided in the technical data sheet and are to be used for acceptance of the data obtained. These reagents are frequently ready to use without dilution and may also be lyophilized. Alternatively, IQCs may be obtained from providers independent of the immunoassay manufacturer, possibly requiring adapted dilutions. Lastly, the CDL may use single or pooled patient samples. In the latter two situations the CDL has to determine the target value of the respective IQC. We analyzed if the CVs of the IQCs are different depending on the source of the IQC sample (Fig. 3). We observed that lower CVs were obtained with commercial controls when compared to IQCs derived from in-house samples, especially for the intra-run CVs ($p < 0.0001$).

3.3. Determination of acceptable precision performance

Acceptable precision performance was calculated for each autoantibody according to the levels of the IQC. The highest CV values were obtained with low level IQCs (< 2 fold the positive threshold). CV values also depend on the type of autoantibody measured. In particular, assays for anti-CL or anti-B2GP1 autoantibodies have higher CVs. Irrespective of the analyte, the method and the level of the IQC, the 90th percentile precision performance goals (lowest CV values reached by 90% of laboratories) were between 3.3% and 16.2% for the intra-run CVs, and between 6.7% and 23.2% for the inter-run CVs. At the 50th percentile precision performance goal values (lowest CV values reached by 50% of laboratories) were between 2.9% and 10.7% for the intra-run CVs, and between 2.8% and 13.2% for the inter-run CVs (Table 1).

3.4. Impact of the type of immunoassay on CV results

The main types of immunoassays used by participants were fluorescent-enzyme immunoassays (FEIA: 39%), chemiluminescence (CLIA; 26%), enzyme linked immunosorbent assay (ELISA; 23%), addressable

laser bead immunoassay (ALBIA; 7%), and nephelometry/turbidimetry (5%). Other methods (radio-immunoassays or immunocompetition) represented less than 1% of the methods reported. In total twenty suppliers of in vitro diagnostic reagents were represented in the study (supplemental Fig. 1).

We analyzed the results of intra-run and inter-run CVs and calculated the performances at 90th and 50th percentiles according to the type of immunoassay used (Figs. 4 and 5).

This analysis revealed larger distribution of the intra-run CVs (Fig. 4A) and higher CV values with ELISA compared to the other immunoassays (Fig. 4B, range of CVs at 90th percentile: 11.4–13.5% for ELISA vs 3.2–12.2% for the other immunoassays), and especially for the low level IQCs.

Table 2 depicts the 616 results of inter-run CVs collected according to the type of immunoassay and the levels of IQCs. Irrespective of the IQC levels, larger distribution was observed for the inter-run CVs obtained with ELISA (median: 11.06%, IQRs (7.15–15.82)) as compared to the other methods (FEIA: median: 8%, IQRs (6.07–10.71); CLIA: median: 6.78%, IQRs (4.1–9.67); ALBIA: median: 7.8%, IQRs (6.1–9.19); nephelometry/turbidimetry: median: 2.55%, IQRs (1.97–5.37)) (Fig. 5A).

Moreover, for the 90th percentile values, higher CVs of intra- and inter-run CVs were observed for low level IQCs (mean 10.9% and 16.2%, respectively) as compared to high level IQCs (8.2% and 13.8%, respectively, $p = 0.008$ and 0.09 , unpaired t test), irrespective of immunoassay type.

We then analyzed the impact of the type of immunoassay for specified autoantibodies on precision performance. We observed significant differences in inter-run CVs for IgG anti-CL assays, with higher values observed with ELISA as compared to FEIA, CLIA and ALBIA. For RF, significantly lower values were observed with nephelometry/turbidimetry as compared to FEIA and ELISA. For the other autoantibodies low or no differences were observed (Mann and Whitney, supplemental data, Fig. 2).

4. Discussion

This article presents the results of a study proposed to French CDL, aiming to collect and analyze the CVs of intra-run and inter-run

Table 1

90th and 50th percentiles CVs for each analyte, each level of IQC and for intra- and inter-run variation. The colors indicate the lowest (in green) and the highest (in red) values of acceptable CVs.

	CV90 (90th percentile)						CV50 (50th percentile)					
	Intra-run CV (%)			Inter-run CV (%)			Intra-run CV (%)			Inter-run CV (%)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
RF	10.0	8.2	8.8	13.2	14.3	11.0	3.8	3.9	3.9	2.8	6.4	6.9
Anti-dsDNA	10.7	6.5	7.5	19.6	12.9	15.9	5.7	4.6	4.6	13.2	7.9	7.0
Anti-TG	14.5	10.1	10.9	12.3	10.0	16.0	4.3	6.8	3.9	6.6	4.7	5.8
Anti-TPO	7.7	6.9	7.0	14.0	18.0	9.4	5.0	4.7	4.0	10.2	7.4	3.9
Anti-TSH-R	10.0*	3.3*	7.0*	10.7*	10.7*	10.7*	8.0*	10.7*	7.0*	7.8*	7.8*	7.8*
Anti-TTG IgA	16.2	6.0	6.2	15.2	13.4	11.8	5.5	4.6	5.1	8.4	8.8	6.8
Anti-B2GP1 IgM	7.8	5.4	10*	13.6	6.7	13.0	3.3	2.9	7.0*	9.2	4.4	10.5
Anti-B2GP1 IgG	10.6	6.2	6.9	23.2	13.0	11.6	5.7	5.3	3.0	11.2	6.1	8.0
Anti-CL IgM	7.9	10.6	6.0*	20.2	11.1	15.0	3.1	7.4	5.0*	7.9	6.7	10.8
Anti-CL IgG	11.5	12.4	12.6	20.3	16.1	17.4	5.0	6.7	8.1	9.4	7.9	8.0
Anti-CCP IgG	11.6	6.7	7.8	14.8	11.8	21.3	4.5	4.8	5.0	7.8	7.4	9.3
MPO-ANCA	9.9	10.3	6.2	13.3	15.1	10.9	5.2	3.9	4.0	10.0	8.5	7.5
PR3-ANCA	12.3	10.5	11.0	14.9	15.4	12.1	6.0	3.4	7.0	10.4	9.2	8.0
Anti-GBM	11.7	8.0*	6.3	16.4*	16.4*	16.4*	4.1	8.0*	5.2	10.1*	10.1*	10.1*

*Results calculated from less than 5 values.

RF: rheumatoid factor, dsDNA: double stranded-DNA, TG: thyroglobulin, TPO: thyroperoxidase, TSH-R: TSH-receptor, TTG: tissue transglutaminase, B2GP1: beta 2 glycoprotein 1, CL: anti-cardiolipin, CCP: cyclic citrullinated peptides, MPO: myeloperoxidase, PR3: proteinase 3, ANCA: antineutrophil cytoplasmic antibodies, GBM: glomerular basement membrane.

A. **B.**

	CV90 (90th percentile)			CV50 (50th percentile)		
	Low	Medium	High	Low	Medium	High
FEIA	10.8	10.2	9.8	5.5	6.1	5.5
CLIA	6.8	4.4	3.7	3.2	2.6	2.7
ELISA	13.5	12.8	11.4	8.9	6.1	4.7
ALBIA	12.2	8.6	10.1	6.0	7.1	6.0
Nephelometry/ Turbidimetry	8.9	3.2	—*	1.1	1.0	—*

Fig. 4. (A) CVs for intra-run variation according to the levels of autoantibodies of the IQC and type of immunoassay. Dotted lines indicate acceptable limits corresponding to 50th percentile and plain lines acceptable limits corresponding to 90th percentile. Table (B) resumes the values corresponding to 90th percentile (i.e. the lowest intra-run CV value obtained by 90% of the laboratories). *: Not applicable, only one value reported.

Fig. 5. (A) CVs for inter-run variation according to the levels of autoantibodies of the IQC and type of immunoassay. Dotted lines indicate acceptable limits corresponding to 50th percentile and plain lines acceptable limits corresponding to 90th percentile. Table (B) resumes the values corresponding to 90th percentile (i.e. the lowest inter-run CV value obtained by 90% of the laboratories). *: Acceptable limits calculated from less than 5 values.

variations of assays used for the detection of 14 autoantibodies, obtained in real life conditions. Despite the low rate of responses (36% of the invited laboratories), the data collected allowed the analysis of 414 values of intra-run CV and 616 values of inter-run CV. The difference in the number of data for intra- and inter-run variations is linked to the fact that inter-run variation is a parameter followed by all laboratories

but the determination of intra-run variation is usually performed only for the initial verification of the methods.

The number of measurements used for the CV calculations had no significant influence on the intra- and inter-run CVs of autoantibody assays. This information is very important because the accreditation rules were defined for biochemistry analytes, such as blood electrolyte

Table 2

Statistical description of the Inter-run CVs results, according to the type of immunoassay and the IQC levels.

Method	Level of IQC	Number of values	Median	IQR	Ranges
FEIA	Low	42	9.30	7.2–12.47	4.40–20.1
	Medium	117	7.42	5.98–10.45	2.90–33.3
	High	75	8.00	6.00–10.30	3.36–32.95
	Low	57	7.73	6.15–10.82	2.39–10.82
CLIA	Medium	69	6.68	4.78–9.91	1.40–14.56
	High	41	3.70	3.46–7.19	1.90–13.5
	Low	33	14.6	11.28–20.93	1.40–36.6
ELISA	Medium	69	10.05	5.98–14.90	1.50–23.00
	High	53	10.00	6.77–15.00	3.58–26.40
	Low	5	5.10	4.1–12.71	4.10–14.61
ALBIA	Medium	16	7.40	6.10–8.93	5.10–12.66
	High	9	7.95	7.42–10.53	4.90–13.50
	Low	16	2.38	2.16–4.11	1.80–14.9
Nephelo/Turbidimetry	Medium	8	1.77	1.72–7.64	1.70–17.00
	High	6	4.07	3.49–7.59	1.97–8.60

FEIA: fluorescent-enzyme immunoassay, CLIA: chemiluminescent immunoassay, ELISA: enzyme-linked immunosorbent assay, ALBIA: addressable laser bead immunoassay, IQC: internal quality control; IQR: Inter-quartile ranges.

concentration, for which low levels of variation may have important clinical significance. Most of the accreditation guidelines have applied these rules to all laboratory specialties without any adaptation according to the clinical significance, the cost-effectiveness, or even feasibility. Indeed the cost for measuring an autoantibody is considerably higher than the measurement of blood electrolytes [11]. In France, the accreditation technical guidance for the use of IQC, recommends calculating the inter-run variability during at least 15 days of measurements, or at least 30 values [14]. Based on our results we conclude that > 10 measurements for either intra- or inter-run CVs will not improve the estimation of the assay precision, whatever the type of immunoassay used. These findings could substantially reduce cost of achieving accreditation if the criteria was reduced from a minimum of 30 observations to 10, if our findings are validated by other studies.

Our study also revealed an impact of the source of the IQC on intra- and inter-run CVs, with lower values obtained when using commercial IQC as compared to IQCs derived from in-house patient samples. This effect doesn't seem to be explained by a biased use of the type of controls according to the method (Supplemental data, Fig. 3). Commercially produced IQCs have the advantage of long term stability and low vial to vial variability. However, the matrix effect on the measurement of analytes may be different from a patient serum and could give erroneously low CVs, which could be detrimental for the interpretation of some patient results, mainly for quantitative assays. For autoantibody assays, this is of high importance considering that the serum to be tested contains high concentrations of IgG, IgM and IgA being non-specific for the autoantigen but potentially influencing the interaction of the antigen-specific autoantibodies to their target. In addition, natural autoantibodies of IgM or IgG isotype may interfere in these immunoassays and their effect on precision should be taken into account [15]. In our analysis we also differentiated data from IQC included in the assay versus IQC independent of the assay manufacturer. Indeed commercial IQC should be bought from a manufacturer different from the assay's provider to ensure an independent assessment of performance [14,16,17]. Moreover, positive and negative controls included in commercial assays are frequently ready to use and thus are not treated in the same manner as patient samples. This type of IQC does not reflect the exact uncertainty of measurement and potentially provides a false acceptance limit. For all of these reasons, the use of patient-derived IQC samples has been recently recommended for antinuclear antibody testing by indirect immunofluorescence [18].

Regarding the type of immunoassays, we noticed an over-representation of the fully automated methods, mainly FEIA and CLIA which represented more than half of the immunoassays. This has a significant impact on the proposed acceptable performance, mainly on the 50th percentile performance (inter-run CV lower than 9% at low levels vs 14.6% for ELISA).

To investigate the differences observed between the 50th and the 90th percentile performances, we performed sub-analyses of the results of autoantibody assays according to the immunoassay method. No major difference in performance according to the type of immune-assay was observed except for RF and anti-CL autoantibodies for which higher CVs were observed when using ELISA, as compared to the other types of immunoassay. This difference is not explained by the use of different kinds of automatization or of manual techniques for the same parameter because the data didn't demonstrate lower CVs obtained by manual techniques (data not shown). In addition, this phenomenon seems particularly important when the IQC are at low concentration levels. Indeed, our results show clearly the higher imprecision when the autoantibody levels of the IQC are below 2-fold the positive threshold of the assay. When studying the 90th percentile values, the highest CVs of intra- and inter-run CVs were also observed with low level IQCs. The imprecision observed with low level IQCs is part of the so-called “grey zone” of the autoantibody assays. This zone corresponds to borderline positivity and may have a significant effect on the patient diagnosis and care. That's why it is very important to be aware of the assay

performances at these levels. For the diagnosis or classification of several autoimmune diseases, guidelines propose using serological criteria of relatively high autoantibody levels. This applies for the anti-phospholipid syndrome [19], rheumatoid arthritis [20] and coeliac disease [21].

The determination of the positive cut-off, and consequently of the grey-zone, remains debated for autoantibody detection. Indeed, their presence and levels in the normal population don't fit a Gaussian distribution and their prevalence may vary from one autoantibody to another [22]. Due to this imprecision a commentary to the physicians should be added to the results within the “grey zone”, in which measurement uncertainty is very high. Indeed, the laboratory should give information about the precision allowing appropriate interpretation of each patient result, at least at the clinically relevant levels.

No reference values for intra- and inter-run CVs are available for autoantibody detection assays in biorepositories. Some data are present on the Westgard website for anti-TPO and anti-TG autoantibodies and for RF, but they are based on only two studies with very few data (33 and 34 samples, respectively). These studies didn't really measured the intra- and inter-run CVs of these parameters but rather the biological variation observed in different samples collected from the same individuals during a short period of time. These values of within-subject variations (anti-TG: 8.5%, anti-TPO: 11.3%, RF: 8.5%) don't inform specifically about the analytical performances of the assays per se [23,24]. In addition, they are of limited interest in the field of autoimmunity in which for some autoantibody, the levels may vary quickly in patients with active disease.

In conclusion, our study revealed that autoantibody measurements exhibit higher imprecision than those observed in clinical chemistry and hematology. However, the imprecision of the autoantibody assays that we report here, is not prejudicial for patient care according to the different clinical guidelines. Though variable according to the different autoimmune diseases, such as in coeliac disease, lupus or vasculitis, [25–28] the significant clinical variations, i.e levels variation that have an impact on the patients diagnostic, prognosis or management, are usually higher than the imprecision of the assays. However, it would be useful for the physicians to be informed about the measurement uncertainty inherent to each autoantibody assay.

This study allows us to propose that an acceptable performance for intra- and inter-run CVs should be below the 90th percentile. Robustness of an assay should thus be re-evaluated if CVs are above the 90th percentile value.

Similar larger studies should be performed by international societies in the field of autoimmunity in order to validate our findings. Nevertheless, regarding the absence of any available data, our work represents a first step in the analysis of proficiency testing of the CDL implicated in autoantibody diagnostics.

Recommendations:

- IQC must be defined in terms of source and levels of autoantibody according to the positive threshold of the assay.
- IQC should comprise at least one positive sample obtained from a source different from the manufacturer of the assay.
- IQC should comprise at least one positive sample that is to be treated in an identical manner as patient samples.
- Intra- and inter-run CVs should be comprised between the 50th and 90th percentile provided by this study; robustness must be re-evaluated if they are > 90th.
- Intra- and inter-run CVs should be calculated from at least 10 values
- The precision of the autoantibody assays must be adapted to their clinical significance and their results must be accompanied by appropriate biological comments.

Statement of author contributions

Marie Senant collected and analyzed the data. All authors

participated in the design of the study, the drafting of the manuscript and provided approval of the final submitted version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clinbiochem.2020.05.019>.

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